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Zoological Research

Effects of Three Plant Powders on Behaviour, Mortality and Reproductive Fitness of *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae)

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Abstract: The effects of powders from dry flower buds of *Eugenia aromatica* Baill., seeds of *Piper guineense* Schum and Thonn and fruits of *Capsicum frutescens* L. on adult behaviour, mortality and reproductive fitness of the cowpea seed beetle, *Callosobruchus maculatus* (Fab.) were investigated under ambient laboratory conditions. All experiments were carried out in glass Petri plates. All powders elicited aversion in adult beetles. The contact toxicity symptoms included restlessness, loss of coordination, knock down and eventual death of adult beetles. These behaviours were more pronounced with *E. aromatica* in which adult beetles died within 16 hours. *P. guineense* and *C. frutescens* powders did not cause 100% mortality of adult beetles, even after 24 hours. Each of the three powders significantly ($P < 0.05$) reduced the mating competition of adult males after sub-lethal exposure for one, two, and three hours, respectively. *E. aromatica* powder caused more reduction in male mating competition for females than any of the other two powders after each period of exposure. Receptiveness of treated females to courting males was also decreased by exposure to any of the three powders. Similarly, *E. aromatica* powder caused greater decreases in female receptiveness to males than any of the other two powders after each period of exposure. Exposure of either adult male or female *C. maculatus* to the powders for sub-lethal times of three, six and nine hours significantly reduced the fecundity of the beetles. *E. aromatica* powder caused the most reduction of eggs laid and also significantly reduced fertility of the eggs.

Key words: *Callosobruchus maculatus*; Plant powder; Behaviour; Mortality; Reproductive fitness

三种植物粉剂对四纹豆象(鞘翅目:豆象科)行为、死亡率和繁殖的影响

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关键词: 四纹豆象; 植物粉剂; 行为; 死亡率; 繁殖率

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The cowpea seed beetle, *Callosobruchus maculatus* (Fabricius) is a serious storage pest of cowpeas, *Vigna unguiculata* (L.) Walp, which are dietary staples in the West African Sahel (Jackai & Daoust, 1986). The edible seeds of cowpeas are the cheapest source of protein in human diets in the region. This is very important because the majority of the populace cannot afford the usually expensive protein from animal sources. Cowpea seeds damaged by *C. maculatus* are riddled with exit holes of adult beetles and have reduced weight, low market value and poor viability. When severely damaged, cowpea seeds become mouldy and unfit for human consumption. It has been estimated that about 4% of the total annual production of cowpea valued at over 30 million US dollars is being lost due to the cowpea seed beetle in Nigeria (Singh, 1986).

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More recent surveys of cowpea in markets and village stores in West Africa indicated that 20 – 90% of seeds might be holed by storage beetles (Alebeek, 1996; Ogunkoya & Ofuya, 2001). There is therefore no doubt that serious depredation of cowpea seeds by *C. maculatus* can threaten food security and the alleviation of poverty in the West African Sahel (Ofuya, 2003).

Synthetic insecticides which are effective in the control of *C. maculatus* in cowpea are available, but their widespread use in developing countries is hampered by high costs, inconsistent supplies and concern for the environment and human health (Ofuya, 2003). Studies by developing country scientists in the past two decades have considered the possibility of finding plant-derived insecticides as alternatives to synthetic chemicals in the control of *C. maculatus* (Lale, 1995; Boeke et al, 2001). Plant-derived materials are thought to be environmentally friendly, more readily available and affordable (Jackai & Daoust, 1986). In Nigeria, the efficacy of many insecticidal plant powders has been investigated for reducing damage by *C. maculatus* to stored cowpea (Ivbijaro, 1983; Ivbijaro & Agbaje, 1986; Lale, 1994; Ogunwolu & Idowu, 1994; Ogunwolu & Odunlami, 1996; Adedire & Lajide, 2001; Ofuya & Dawodu, 2002a, b; Ofuya & Salami, 2002). However, studies on the effects of insecticidal powders on the behaviour, mortality and reproductive fitness of the seed beetles have not been adequately considered. Such studies will enable us to elucidate the modes and mechanisms of action of these plant powders.

In this paper, the results of investigations into the effects of powders from dry flower buds of *Eugenia aromatica* Baill, seeds of *Piper guineense* Schum and Thonn and fruits of *Capsicum frutescens* L. on adult behaviour, mortality and reproductive fitness of *C. maculatus* under ambient laboratory conditions are presented.

1 Materials and Methods

1.1 Insect culture

The *C. maculatus* used in this study was from a laboratory colony of an Akure, Nigeria population regularly maintained in the Crop, Soil and Pest Management Research Laboratory, at Obanla campus of the Federal University of Technology, Akure, Nigeria. In maintaining this colony large numbers of individuals were introduced to 200 g of clean "Ife brown" cowpea (a well known susceptible variety) in clear plastic containers with meshed lids every month. The colony had been maintained this way for over 40 generations. It is not known whether laboratory and natural populations would respond differently to applied control measures. All subsequent experiments were carried out using "Ife brown".

1.2 Preparation of insecticidal powders

Dry flower buds of *E. aromatica* Baill, fruits of *P. guineense* Schum and Thonn, and *C. frutescens* L. were purchased from local herbal stores in Akure, Nigeria. Their identities were confirmed at the Herbarium at the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. They were again oven-dried at 60 ℃ for 48 hours, ground to a powder in an electric mill, and sieved with a 300 μm mesh. Each powder was stored in an air-tight polyethylene bag until used less than a month later.

1.3 Behaviour and mortality of adult beetles treated with powder

The behaviour and mortality of freshly emerged (0 – 24 hours old) adult *C. maculatus* exposed to each powder were observed in clean glass Petri plates (9.0 cm diameter) under ambient laboratory conditions. Twenty adults (10 males and 10 females) were put on a plate and shaken with 0.2 g of powder. The physical responses of the insects to this treatment were observed and noted for four consecutive hours. Adult mortality was observed every four hours for 24 hours. Adults were considered dead when unperturbed by a pin prick. This experimental procedure was repeated five times.

1.4 Mating competition of adult beetles treated with powder

Freshly emerged (0 – 24 hours old) adult *C. maculatus* were placed for different periods of time (1, 3, 6, 9, and 12 hours) respectively on 20 g of cowpea seeds in a Petri plate dusted with 0.4 g of powder. At the end of each time period, a beetle exposed to the powder was removed from the treated seeds, marked (with a black point) and matched with another adult of same sex on cowpea seeds not dusted with powder. In order to determine male mating competition, an unexposed female was placed in a clean Petri plate and the exposed and the matching unexposed males added to the plate. Competition was judged by the number of times each adult male (exposed and unexposed) gained the copulation position (mounted the female) within different periods of observation (1, 2, and 3 hours, respectively). After each mounting, the successful male was quickly dislodged by prodding with camel hair brush. A successful mounting was that the aedeagus of

the male has made contact with the female. The comparative male receptiveness of females exposed and unexposed to each powder was determined according to the procedures outlined for males. Mating competition was determined for 10 individuals (exposed and unexposed) of either sex and for each powder.

1.5 Fecundity and fertility of adult beetles treated with powder

Freshly emerged (0 - 24 hours old) adult *C. maculatus* were placed for different periods of time (1, 3, 6, 9, and 12 hours) respectively on 20 g of cowpea seeds in a Petri plate dusted with 0.4 g of powder. At the end of each time period, a beetle exposed to the powder was removed from the treated seeds, and transferred into another Petri plate containing 20 g of clean cowpea seeds and an unexposed adult of same age but of opposite sex. The number of eggs laid on the seeds was counted when the insects died. The numbers of hatched and unhatched eggs were also noted. Hatched eggs were defined by the presence of larval frass, which causes eggs to turn milky-white as larvae bored into the seed (Giga & Smith, 1987). Unhatched eggs remain transparent and glossy. Each treatment was replicated 10 times.

1.6 Data analysis

Data generated in each experiment were separately subjected to analysis of variance. Percentages were arcsine transformed before analysis. Significant means were identified using appropriate statistics.

2 Results

2.1 Behaviour and mortality of adult beetles treated with powder

The behaviour of adults exposed to powders of *P. guineense* and *C. frutescens* were almost similar. After shaking the contents of the Petri plate, the adult beetles immediately moved away from the bottom where the powder concentrated, and clung to the inner wall of the lid. A few attempts at copulation were made. After about forty minutes the beetles were dropping from the wall and lid roof and climbing up again. Their movements were slow and irregular. There was continuous beating of their hind limbs and rapid movements of the antennae. These behaviours continued for another two hours. During this period no copulation attempts were observed. At about four hours after treatment some beetles were already very weak and lying flat on their backs and shaking their limbs. Some beetles were moving on their backs with the hind limbs tilted as if to regain normal position. At this period many of them attempting to climb to the wall and lid roof did not succeed.

The adult beetles also immediately displayed strong aversion to the powder of *E. aromatica*. Within five minutes of contact, adults exhibited restlessness manifested as fast irregular movements. Flexing of wings was noticed in male bruchids. Attempts at copulation were scanty. After treatment for fifteen minutes, some adults were already partially immobilized. They were seen flexing their wings without change in position. No attempts at copulation were made. After treatment for thirty minutes, many adults had experienced total immobilization. Others displayed extreme weakness with very slow movement when prodded with a camel hair brush. After treatment for one hour, many individuals had seemingly become lifeless and lay on their backs. They remained in this condition for many hours. Sign of life was observed only after intense prodding with a camel hair brush.

E. aromatica powder caused significant mortality of adult beetles exposed to it for four hours (Tab. 1). Death of the adult beetles was achieved 16 hours post-treatment. In contrast, *P. guineense* and *C. frutescens* powders did not cause 100% mortality of adult beetles in 24 hours.

2.2 Mating competition of adult beetles treated with powder

Irrespective of period of exposure, each of the three powders significantly ($P < 0.05$) reduced both mating competition of males for females and female receptiveness for males in *C. maculatus* in comparison

Tab. 1 Mortality of freshly emerged adults of *Callosobruchus maculatus* exposed to three plant powders at different time points (mean ± *SE*, %)

Plant powder	Time point(h)				
	4	8	12	16	24
Eugenia aromatica	14.0 ± 1.87^a	24.0 ± 1.87^a	65.0 ± 3.53^a	100.0 ± 0.00^a	100.0 ± 0.00^a
Piper guineense	0.0 ± 0.00^b	0.0 ± 0.00^b	7.0 ± 1.22^b	14.0 ± 1.87^b	16.0 ± 1.87^b
Capsicum frutescens	0.0 ± 0.00^b	0.0 ± 0.00^b	2.0 ± 1.22^b	6.0 ± 1.87^c	9.0 ± 1.87^c
Control	0.0 ± 0.00^b	0.0 ± 0.00^b	0.0 ± 0.00^b	0.0 ± 0.00^d	0.0 ± 0.00^d

Means in each column bearing different letters are significantly different at the 5% level of probability by LSD test.

with the control (Tab. 2). *E. aromatica* powder exhibited stronger effects on *C. maculatus* than the other two.

2.3 Fecundity and fertility of adult beetles treated with powder

Egg deposition by *C. maculatus* was significantly ($P < 0.05$) influenced by exposure of the male in the couple to varying lengths of time to the three plant powders (Tab. 3). Only *E. aromatica* powder significantly reduced the number of eggs laid by *C. maculatus* when the male in the couple was exposed to it for one hour in comparison with the control. Similar results were obtained for all the three powders when the male was exposed for three or more hours.

None of the powders significantly reduced the number of eggs laid by *C. maculatus* when the female in the couple was exposed to them for one hour in comparison with the control (Tab. 3). However, all the three powders significantly reduced the number of eggs laid by *C. maculatus* when the female in the couple was exposed for three or more hours. *E. aromatica* powder significantly reduced number of eggs laid by *C. maculatus* when either the male or the female in the couple was exposed to it more than any of the other two.

The percentage of hatched eggs when adult male or female of *C. maculatus* were exposed for one hour to each of the three powders was not significantly reduced ($P > 0.05$) in comparison with the control (Tab. 4). Only *E. aromatica* powder significantly reduced the percentage of hatched eggs in *C. maculatus* when the male or the female in the couple were exposed to it for three or more hours.

Tab. 2 Number of mountings of *Callosobruchus maculatus* exposed to three plant powders at different period of exposure time (mean ± *SE*, %)

Period of exposure(h)	Male and Female	*Eugenia aromatica*	*Piper guineense*	*Capsicum frutescens*	Control
1	Male	4.6 ± 0.22^a	5.3 ± 0.26^a	6.7 ± 0.30^b	9.7 ± 0.21^c
	Female	5.5 ± 0.43^a	7.4 ± 0.37^b	8.3 ± 0.37^b	9.2 ± 0.33^b
2	Male	2.4 ± 0.16^a	4.8 ± 0.25^b	5.7 ± 0.21^b	10.0 ± 0.33^c
	Female	4.6 ± 0.34^a	5.8 ± 0.33^b	7.1 ± 0.23^c	8.9 ± 0.23^d
3	Male	2.0 ± 0.25^a	4.3 ± 0.30^b	4.9 ± 0.28^b	10.0 ± 0.33^c
	Female	3.2 ± 0.25^a	5.4 ± 0.22^b	6.4 ± 0.22^c	8.6 ± 0.16^d

Means in each row bearing different letters are significantly different at the 5% level of probability by LSD test.

Tab. 3 Egg deposition by *Callosobruchus maculatus* as influenced by exposure of male or female in the couple for varying lengths of time to three plant powders (mean ± *SE*, %)

Plant powder	Male and Female	Time of exposure(h)			
		1	3	6	9
Eugenia aromatica	Male	23.4 ± 2.67^a	11.2 ± 3.39^a	2.7 ± 1.10^a	0.0 ± 0.00^a
	Female	57.1 ± 5.51^a	13.7 ± 2.82^a	7.4 ± 1.01^a	7.1 ± 1.40^a
Piper guineense	Male	67.9 ± 6.70^b	33.1 ± 4.62^b	27.2 ± 3.66^b	16.1 ± 2.59^b
	Female	74.9 ± 5.89^a	46.1 ± 2.88^b	26.6 ± 3.25^b	23.1 ± 3.52^b
Capsicum frutescens	Male	70.8 ± 6.70^b	43.1 ± 3.58^b	25.3 ± 3.09^b	22.8 ± 2.25^b
	Female	72.0 ± 6.47^a	42.8 ± 3.85^b	28.9 ± 3.87^b	23.6 ± 2.66^b
Control	Male	69.4 ± 5.51^b	69.4 ± 5.51^c	69.4 ± 5.51^c	69.4 ± 5.51^c
	Female	69.1 ± 3.80^a	66.9 ± 3.47^c	66.9 ± 3.47^c	66.9 ± 3.47^c

Means in each column bearing different letters are significantly different at the 5% level of probability by Tukey's test.

Discussion:

Several normal behavioural mannerisms have been observed in *C. maculatus* during pre-oviposition, mating, oviposition and post oviposition periods in the life history of the beetles (Rup, 1986; Parr, 1994; Ofuya, 1995; Ofuya & Adenekan, 1997). These behavioural patterns appeared to be disrupted when adult beetles were exposed to the insecticidal powders in this study. For instance, males not treated with powder ceaselessly pursued females, but males exposed to any of the powders pursued females less frequently and with less agility. Movements of treated males and females were uncoordinated and frequently staggering. In addition, *E. aromatica* treated males frequently flexed one or both wings. Restlessness and loss of coordination of adult *C. maculatus* upon exposure to powder of *Zanthoxylum zanthoxyloides* (Lam.) Waterm has been reported (Ogunwolu et al, 1998).

Tab. 4 Fertility in *Callosobruchus maculatus* as influenced by exposure of male or female in the couple for varying lengths of time to some plant powders (mean ± *SE*, %)

Plant powder	Male and Female	Time of exposure(h)			
		1	3	6	9
Eugenia aromatica	Male	83.9 ± 1.87	58.3 ± 2.98^{a}	33.0 ± 2.41^{a}	19.8 ± 1.96^{a}
	Female	80.2 ± 3.66	40.3 ± 2.66^{a}	30.1 ± 2.44^{a}	20.4 ± 2.58^{a}
Piper guineense	Male	85.1 ± 1.79	84.1 ± 1.76^{b}	84.3 ± 1.80^{b}	82.9 ± 1.60^{b}
	Female	85.0 ± 1.90	84.6 ± 1.72^{b}	83.3 ± 1.88^{b}	81.6 ± 1.62^{b}
Capsicum frutescens	Male	83.7 ± 1.73	83.5 ± 1.65^{b}	83.9 ± 1.71^{b}	82.7 ± 1.54^{b}
	Female	84.9 ± 1.77	84.5 ± 1.64^{b}	85.6 ± 1.68^{b}	85.2 ± 1.49^{b}
Control	Male	84.1 ± 2.62	84.1 ± 2.62^{b}	84.1 ± 2.62^{b}	84.1 ± 2.62^{b}
	Female	86.1 ± 1.81	86.1 ± 1.81^{b}	86.1 ± 1.81^{b}	86.1 ± 1.81^{b}

Means in each column bearing different letters are significantly different at the 5% level of probability by LSD test.

The speed at which adults of *C. maculatus* were killed by the three powders was consistent with observations by earlier works (Adedire & Lajide, 2001; Dawodu & Ofuya, 2000). It was observed that at the generally effective rate of application of 2% of stored grain (Lale, 1995), the powder of *E. aromatica* could kill adult *C. maculatus* in 16 hours.

The modes of action of these plant powders, especially *E. aromatica* which rapidly killed adult *C. maculatus* could be speculated from the recorded observations. Symptoms such as restlessness, hyperexcitability, rapid induction of unconsciousness, paralysis and death in adult beetles caused by *E. aromatica* powder suggested that the nervous system was adversely affected. This is the mode of action of most conventional insecticides (Pedigo, 1991). The fact that *E. aromatica* powder has strong fumigant action against adult *C. maculatus* (Ofuya, unpublished data) lends credence to the nervous system disruption hypothesis. Products of some plants like *Ryania speciosa* L. and *Schoenocaulon officinale* L. disrupts the insect muscular system resulting in increasing oxygen consumption, followed by flaccid paralysis and death (Pedigo, 1991). Ogunwolu et al (1998) and Adedire & Ajayi (2003) also suggested that some plant-derived insecticidal materials could cause respiratory impairment which could lead to loss of strength and vigour, and ultimately death of the insect. Detailed morphological, anatomical, physiological and biochemical studies of male and female *C. maculatus* exposed to these plant powders are required to provide further empirical information.

It was reported that sub-lethal amounts of harmful agents could induce enhanced reproduction in some pest insects through the phenomenon of hormoligosis (Pedigo, 1991). For instance, sub-lethal amounts of citronella and clove oils caused female *C. maculatus* to lay more viable eggs or improve the hatching of eggs laid on oil-treated seeds (Lale, 2002). Hormoligosis was not observed in this study. At best, exposure of female *C. maculatus* in a couple to any of the three insecticidal powders for a sub-lethal time of one hour did not significantly influence fecundity. Otherwise, exposure of either sex of the beetle to the powders for sub-lethal times of 3, 6 or 9 hours caused significant reduction in fecundity. Exposure of male or female *C. maculatus* to powder of *E. aromatica* for a sub-lethal time of 3, 6 or 9 hours also significantly reduced the hatching of eggs laid. Ofuya (1990) postulated that weakening of adults by insecticidal plant powders might cause them to lay fewer eggs. The same mechanism may have been operative in this study. It was observed that males exposed to each of the three plant powders mated less frequently than unexposed individuals. It was reported that an increase in the number of copulations increased fecundity and ovarian production of females of *C. maculatus* (Ofuya, 1995). The reduced fertility of eggs laid by *C. maculatus* when male or female in a couple was exposed for sub-lethal time to *E. aromatica* powder may be explained by at least two possibilities. First, the powder may have a sterilizing effect on the beetles. Second, the weakened male may have been incapable of normal copulation and ejaculation.

Fatty acids, phenolics, alkaloids and terpenes, especially monoterpenes, are often found to be the bioactive constituents of plant products (Lale, 1995). Dales (1996) compiled some of the toxic chemicals found in *Eugenia/Syzigium*, such as tannic acid, α-pinene, methyl salicylate, methyl eugenol, methol, linallol, limonene, geraniol, furfural, eugenol, cineole (1 - 8), and carvone. *Piper* species contained anethole, apiole, asarone, carene, cineole, citral, dihydrokawain, eugenol, eugenol methly ether, hydrocyanic acid, limonene, phellandrene, piperidine, piperonal, safrole and stearyl alcohol (Dales, 1996). The bioactivity of *C. frutescens* is ascribed to the presence in the fruit of a volatile phenolic compound, capsaicin (Lale,

1995). Thus, the constituent chemicals in the powders used in this study may be different, and may partly explain their different levels of activity on *C. maculatus*.

Overall, this study elaborated some components of the protective ability of the three plant powders examined against infestation and damage to stored cowpeas by the notorious *C. maculatus*. Male and female beetles mate soon after emergence and the female normally lays approximately 45 per cent of the eggs within the first day of life (Wasserman, 1985). Thus, the ability of *E. aromatica* powder to terminate adult life of the beetle in 16 hours is a major factor in its protective ability. The loss of male mating competition and female receptiveness could be expected to reduce population growth of the storage beetle. Failure to mating results in deposition of infertile eggs (Ofuya, 1995; Ogunwolu et al, 1998), culminating in fewer beetles. The reduced fecundity caused by the powders would also reduce beetle population growth. Finally, the reduced fertility of beetle eggs caused by exposure of adults to the powder of *E. aromatica* will still further reduce beetle population growth.

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